Technical
User Guide

ExoHERO®1
Product Code: EH0001001A

ExoHERO®10
Product Code: EH0010001B

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PURITY by DESIGN

IMPORTANT NOTES

- Read this technical user guide before using the capsule.
- Exo**HERO**® capsules contain a storage buffer of 20% ethanol. For long term storage the capsule should be refrigerated between 2ºC (36ºF) and 8ºC (46ºF) and kept away from direct sunlight. For short term storage, the capsule can be kept at room temperature. Keep caps on the capsule until use. Do not freeze.
- This product is provided as non-sterile, unless specified.
- This product is sanitizable, please follow instruction included.
- This product is intended for single batch use. A clean-in-place method is provided. As feedstocks and processes vary, it is recommended that the performance of this product is validated after CIP (clean-in-place).
- The technical information can change without notice. For the latest information please refer to the Astrea Bioseparations website (https://www.astreabioseparations.com/) for the most current version of this document.

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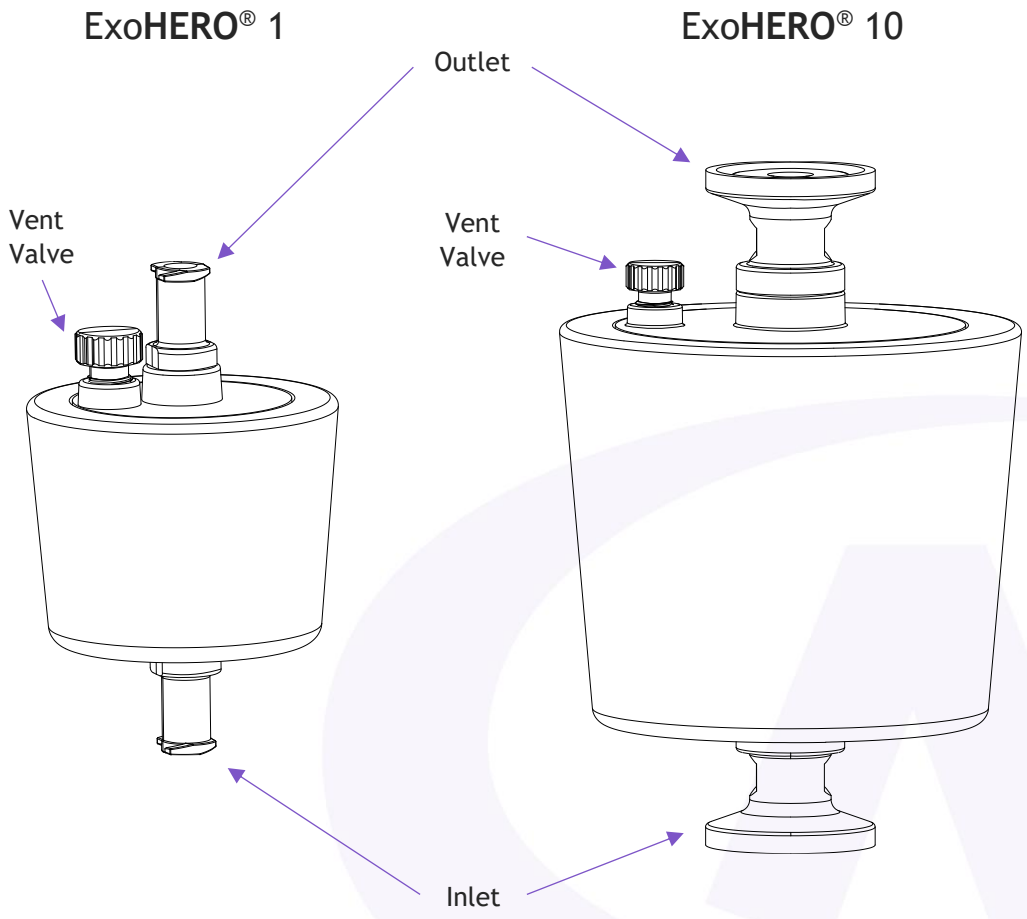
INTRODUCTION

ExoHERO® is a radial pre-packed chromatography capsule designed for downstream processing of extracellular vesicles (EV) from complex feedstock.

ExoHERO® consists of a proprietary high-flow and high-capacity composite nanofiber, AstreAdept®, housed in a protective shell specifically designed to reduce void volume.

The combined characteristic of a large flow path diameter, weak anion exchange chemistry and mild elution conditions enable high recovery of purified EV, including exosomes.

PRODUCT DESCRIPTION



TECHNICAL DATA

SPECIFICATIONS	ExoHERO® 1	ExoHERO® 10
LIGAND:	Weak anion exchange	
TYPICAL DYNAMIC BINDING CAPACITY: Bovine serum albumin at 1 mg/mL in 10 mM at Tris pH 8	>60 mg/mL	
MAXIMUM PRESSURE AT 20°C:	6 Bar | 0.6 MPa | 87 psi	
BED VOLUME (BV):	1 mL	10 mL
NOMINAL VOID VOLUME:	3.5 mL	30 mL
RECOMMENDED OPERATIONAL FLOW RATE (mL/min):	5	50
RECOMMENDED PRE-CAPSULE PRESSURE:	3 Bar | 0.3 MPa | 43 psi	
CHEMICAL STABILITY:	Compatible with buffers commonly used in bioprocessing, including 20% EtOH, 1 M acetic acid, 1 M NaOH, and solutions containing up to 10 mM EDTA	
CONNECTIONS:	Luer	Sanitary flange (12.7 mm)
VENT VALVE:	Screw cap	Screw cap
REPEATED USE:	Single batch use	
MATERIALS OF CONSTRUCTION:	HOUSING: Polypropylene, Silicone FUNCTIONAL MEMBRANE: modified cellulose acetate	

INSTALLATION

WARNING

Do not use high concentrations of organic solvents for example greater than 20% EtOH, or 30% isopropyl alcohol.

Wear appropriate personal protective equipment during operation.

Deviation from recommended guidelines could result in personal harm, or damage to the product or material.

Buffer compositions are listed in the Operations section.

Use with a liquid chromatography system

1. Using an appropriate stand (e.g., ELS-UCS-SMALL for Exo**HERO**® 1) securely attach Exo**HERO**® with the vent outlet facing upwards. Attach the Exo**HERO**® to the chromatography system with appropriate tubing. For example, for Exo**HERO**® 1 use green PEEK, 1/16" OD x 0.75mm" ID, and finger tight connectors with the supplied luer connectors.

2. Attach the column outlet position from the chromatography system to the bottom inlet of the Exo**HERO**®, and the column inlet to the top outlet of the Exo**HERO**® and loosen the stopper on the vent.

3. Run water through the system and once liquid is visibly exiting the vent, close the stopper on the bleed valve. Continue to run 5 bed volume (BV) of water through the Exo**HERO**® to remove the storage buffer.

4. If required, run 5 BV of the Sanitizing Solution at 1 BV/min, and then hold for 30 minutes.

5. Equilibrate the Exo**HERO**® with Phosphate Buffer Saline (PBS)) at 1 BV/min until pH and conductivity are stable.

6. Run the High Conductivity Solution through the Exo**HERO**® at 5 BV/min to charge the unit.

7. Re-equilibrate with the low conductivity Equilibration Buffer at 5 BV/min until pH and conductivity are stable.

8. The system is now ready to load clarified feed that has been treated with endonuclease.

Use with a peristaltic pump system

1. Place the peristaltic pump in a clean and stable location.

2. Attach the appropriate tubing into the pump head as per manufacturers recommendations. Ensure the tubing fits securely and without kinks.

3. Test the flow rate of the tubing by priming the pump with 20% EtOH or Sanitizing Solution. Collect the liquid from the outlet into a clean tared container over 60s and record the volume. Adjust the rpm of the pump and retest until the desired flowrate is achieved (1 BV/min).

4. Attach tubing to the bottom inlet of the Exo**HERO**®, and appropriate tubing to the top outlet of the Exo**HERO**®. Ensure the tubing leaving the Exo**HERO**® can connect with appropriate collection tubes or waste collection vessel. Loosen the stopper on the vent.

5. Run water through the system and once liquid is visibly exiting the vent, close the stopper on the bleed valve. Continue to run BV of water through the Exo**HERO**® to remove the storage buffer.

6. If required, run 5 BV of the Sanitizing solution at 1 BV/min, and then hold for 30 minutes.

7. Equilibrate the Exo**HERO**® with the desired buffer (e.g., Tris 50 mM pH 8) at 1 BV/min until pH and conductivity are stable.

8. Run the High Conductivity Solution through the unit at 5 BV/min.

9. Re-equilibrate with a low conductivity Equilibration Buffer at 5 BV/min until pH and conductivity are stable.

10. The Exo**HERO**® is now ready to load clarified feed that has been treated with endonuclease.

Manual use with a syringe (Exo**HERO**® 1)

1. Attach a 20 mL syringe filled with water to the bottom inlet of the Exo**HERO**® 1. Attach appropriate tubing to the top outlet of the Exo**HERO**® 1. Ensure the tubing leaving the Exo**HERO**® 1 can connect with appropriate collection tubes or waste collection vessel. Loosen the stopper on the vent.

2. Once liquid is visibly exiting the vent, close the luer connector on the bleed valve. Run 20 mL of water through the Exo**HERO**® 1 to remove the storage buffer.

3. If required, run 20 mL of the Sanitizing Solution through the Exo**HERO**® 1, and then hold for 30 minutes.

4. Equilibrate the Exo**HERO**® 1 with 20 mL of Equilibration Buffer until pH and conductivity are stable.

5. Run 20 mL of High Conductivity Solution (e.g., 2 M NaCl) through the Exo**HERO**® 1 to charge the unit.

6. Re-equilibrate with a 2 x 20 mL syringes conductivity equilibration buffer, until pH and conductivity are stable.

7. The Exo**HERO**® 1 is now ready to load clarified feed that has been treated with endonuclease.

8. OPERATION

EV feedstocks vary in production method, titer, genetic payload, and contamination profile. All these factors can influence the performance of a chromatography step. **Optimization for use with any feedstock may be required.** A general process is described below as an example.

EXAMPLE PURIFICATION PROCESS FOR EXTRACELLULAR VESICLES

Buffers

Buffer	Composition
SANITIZING SOLUTION:	0.5 M NaOH
HIGH CONDUCTIVITY SOLUTION:	2 M NaCl
EQUILIBRATION:	Phosphate Buffer Saline (PBS)
ELUTION:	1 M NaCl in PBS
STRIP:	1 M NaCl in PBS

Preparation of EV feed

Some EV feeds may require treatment with endonuclease before clarification.

EV clarification will vary according to expression systems. Centrifugation, diafiltration and concentration and clarification with 0.8 µM filters are commonly used.

Chromatography method

1. Install, sanitize, equilibrate, charge and re-equilibrate the Exo**HERO**® as described above Installation section.

All steps (except where noted) are run at 5 bed volumes (BV)/min.

2. Load clarified feed to approximately 1.75E+12 EV particles /mL of adsorbent, as measured by nanoparticle tracking analysis.

3. Wash with 30 BV of equilibration buffer.

4. Elute with a gradient up to 100% elution buffer over 30 BV of elution buffer, collecting the elution peak by UV absorbance, to establish where the target elutes (this is expected around 30 to 40 % elution buffer).

5. Clean in place with 10 BV of Strip buffer.

6. Re-equilibrate with 10 BV of equilibration buffer.

7. Sanitize with 30 BV of 0.5 M NaOH at 1 BV/min.

The Exo**HERO**® can now be disposed of safely or re-equilibrated for use with the same target.

STORAGE CONDITIONS

Store capsule between 2ºC (36ºF) and 8ºC (46ºF) away from direct sunlight. Keep caps on the capsule until use. Do not freeze.

Exo**HERO**® is designed for single batch use. However, if storage is necessary the Exo**HERO**® should be cleaned as described in section 5.2 and stored in 20% EtOH at 4ºC. Do not freeze. It is recommended that Exo**HERO**® performance is validated after storage.

TROUBLESHOOTING

Problem	Probable cause	Action
High back pressure during loading	Incomplete clarification	Repeat clarification
EV fail to bind to the Exo**HERO**®	Conductivity of load too high	Dilute load with equilibration buffer until conductivity is approximately 15mS/cm
Early breakthrough of EV	Competitive binding with other impurities	Check dsDNA contamination in feed.

QUALITY ASSURANCE

The product meets the standards as described below.

COMPLIANCE OF MATERIALS:	All wetted parts have been assessed for low toxicity compliance and bio-compatibility against either USP VI <88>, ISO 10993, 21CFR Part 177 or by risk assessment.
DECLARATIONS OF SUITABILITY:	TSE/BSE free, Non-Animal derived, Nitrosamine free statements available
QUALITY:	Manufactured within an ISO 9001:2015 Quality Management System the product is controlled, traceable and released through the Quality function. For research use only.
COMPLIANCE OF PERFORMANCE AND IDENTIFICATION:	Flow test and inspection

ORDER INFORMATION

Code	Description	Pack Size
EH0001001A	Exo**HERO**® 1	1 capsule
EH0010001B	Exo**HERO**® 10	1 capsule

For more information on this or any other supply related matters, please do not hesitate to contact us at sales@astrea-bio.com

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